Fungal endophytes of Himalayan Cold Desert Induces Heat tolerance in Rice (Oryza sativa L.)

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Research Article

Keywords: Cold desert, fungal endophytes, Rice and Heat stress

Posted Date: April 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1339767/v2

Abstract

The plants growing in cold desert of western Himalaya have inhabited diversified endophytes. These endophytes can provide fitness to plant under harsh environmental situations. In the current study, 22 fungal endophytes isolated from Artemisia and Xerophytic plants growing in the cold desert were screened for thermo-tolerance at different temperature ranges (28, 30 32, 34, 36, 38 and 40 o C) under in vitro . The only three isolates viz ., A2, A7 and X5 exhibited growth up to 40 o C and identified as *Penicilium funiculosum* (A2), *Ceriporia lacerate* (A7) and *Endomelanconiopsis endophytica* (X5) using ITS region. These endophytes inoculated to rice seedlings and exposed to elevated temperature (45 °C) for 7hr per day for 10 days to study their effect on tolerance of rice to heat stress. The results revealed that endophytes inoculated seedlings showed sustained improvement in shoot and root growth. The *E. endophytica* was chosen to be the best endophyte to impart heat stress as per Fernandez model. This study suggested that cold desert endophytes could induce heat tolerance in plants.

Introduction

The global temperature increases day by day due to change in climate. Frequent heat waves have had serious impacts on rice production (Zhang and White 2021). Historical data analysis envisaged that 7–8 % of rice yield has been decreased due to raise in temperature to 1^{o}C (Baker et al. 1992). International Rice Research Institute (IRRI) demonstrated that the field trials from 1992-2003 showed 10 % yield reduction of rice for every raise in one degree of minimum temperature (Peng et al. 2004). High temperature affects all stages of rice plant starting from germination, growth, development, reproduction and yield (Krishnan et al. 2011). The tiller number decreased by 10% when temperature rise from 29/21^{o}C to 37/29^{o}C (Manalo et al. 1994). The synchronism between the emergence of main stem and tiller and also mobilization of nutrients among tillers were affected by high temperature resulting in decreased yield as primary tillers are directly proportional to grain yield in rice (Yoshida 1981).

Cold deserts are found in high, flat areas, called plateaus, or mountainous areas in temperate regions of the world. Cold deserts have hot summers but extremely cold winters. The Western Himalayan cold deserts have extremes of hot and cold climate combined with excessive dryness. Soil has light grey, poor in fertility and less water holding capacity. Therefore, these desert plants develop some physiological mechanisms like CAM (Crassulacean acid metabolism), modified leaf and also take the advantage of microbial endophytes to survive in hostile environment (Zhang and White 2021).

The endophytes can colonize the plant tissue without causing any apparent harm and provide fitness under hostile environment. Endophytes can be cultured *in-vitro* and transfer to compatible secondary plants to obtain similar benefits (Baldani et al. 2000; Redman et al. 2002 and Wang et al. 2021). Endophytes isolated from cold deserts seems to adapt wider range of temperature as cold desert has influenced by fluctuated temperature ranges from -45 ^{o}C in winter to 40 ^{o}C in summer (Tewari and Kapoor 2013). Therefore, we used in our study the endophytes isolated from the cold desert plants to understand induction of thermotolerance temperature sensitive rice variety IR-64.

Materials And Methods

Screening for thermotolrance of endophytic isolates

The fungal endophytes isolated from Artemisia and xerophytic plants of Western Himalayan cold desert and preserved at School of Ecology and Conservation Laboratory, University of Agricultural Sciences, Bangalore-560065. The 22 isolates were procured and rejuvenated on potato dextrose agar (PDA) for the present study. The endophytic isolates were screened for temperature tolerance. Isolates were cultured in PDA plates and incubated at different temperature (28 ^{o}C, 30 ^{o}C, 32 ^{o}C, 34 ^{o}C, 36 ^{o}C, 38 ^{o}C and 40 ^{o}C) for five days. Fungal growth was measured by radial diameter of colony on fifth day of incubation.

Molecular identification of thermotolerant endophytic isolates

The endophytic isolates of genomic DNA were extracted by Cetyltrimethylammonium bromide (CTAB) method (Vainio et al. 1998). The internal transcribed spacer (ITS) region of genomic DNA was amplified using universal primer ITS1-F (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4-R (5′ TCCTCCGCTTATTGATATGC 3′) by polymerase chain reaction (PCR). PCR amplification was performed using Master cycler (Eppendorf, Germany) with a 20µl reaction mixture that comprised 2µl 1X taq buffer with $MgCl_2$ (1.5mM), 2µl dNTP’s (10mM), 0.5 µl each primer (10pmol), 0.3µl Taq DNA polymerase (3U) and 1µl template DNA (100ng). The PCR was carried out with an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30s, 55 °C for 1 min and 72 °C for 30s, and a final extension at 72 °C for 12 min. The PCR amplified products were sequenced by SciGenome labs, Cochin, Kerala, India. The nucleotide sequences were queried in the NCBI GenBank database using a Basic Local Alignment Search Tool (BLAST). Sequences of each fungal species and corresponding reference sequences from GenBank were subjected to ClustalW analysis. The phylogenetic tree was constructed through maximum likelihood method and Tamura- Nei model, using MEGA X. The recognized sequences were placed in GenBank with accession number.

Interaction of fungal endophytes with Rice under heat stress

Evaluation of fungal endophytes on their ability to impart heat tolerance in rice (variety IR-64) was carried out in plant growth chamber at Indian Institute of Horticulture Research (ICAR-IIHR), Hesaraghatta, Bangalore. There were two sets of experiments. 1. Heat stress (45 ^{o}C for 7h per day for 10 days) and 2. Without heat stress (normal temperature conditions, 30±0.5 ^{o}C). Each set comprised with following treatments. 1. Control (uninoculated plants) 2.*Ceriporia lacerate* 3. *Endomelanconiopsis endophytica* and 4. *Penicilium funiculosum.* Rice seeds were surface sterilized using 3 % sodium hypochlorite followed by 70 % alcohol. The surface sterilized seeds were repeatedly washed with sterile water and soaked for overnight. The pre-germinated seeds were sown in pots filled with soil and FYM (1:1w/w). Three seedlings per pot were maintained and grown for fifteen days. The thermotolerant endophytes were inoculated by stem prick method (Bhunjun et al. 2020) and allowed to colonize for 10 days. After colonization, set-1 seedlings were exposed to heat (45 ^{o}C) for 10 days in growth chamber. Observations for plant height, number of tillers, number of leaves, root volume, fresh and dry weight of roots were recorded after 10 days of heat exposure. Similarly, observations for plants grown under normal conditions (set-2) were recorded.

Statistical Analysis

The data generated during experimentation was analyzed by one-way analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT) using the software XL STAT. The 3-D plot of stress tolerance index (STI) of biomass was constructed according to Fernandez (1992) model using iPASTIC online tool kit (https://manzik.com/ipastic/).

Results And Discussion

Screening and identification of thermotolerant fungal endophytes

The numerous studies have been conducted on improvement of crop growth under heat stress using thermotolerant endophytes isolated from harsh environment or wild plants. However, the use of cold desert thermotolerant endophytes were less explored therefore we have analysed the effect of cold desert endophytes on improvement of fitness of rice under heat stress. In present study, All endophytic isolates showed good growth up to 30 ^{o}C, beyond that there is gradual reduction in growth. This indicated that the optimum temperature of these isolates ranges from 28 to 30 ^{o}C. Three isolates *viz.*, A2, A7 and X5 recorded tolerance level up to 40 ^{o}C (Table 1). This envisaged that these three isolates could sustain heat stress it might be the cold desert of Western Himalaya had extreme of hot climate (40^{o}C) during summer (Tewari and Kapoor 2013). These endophytes were identified using ITS region of rDNA as *P. funiculosum, C. lacerate* and *E. endophytica* (Fig.1) and the obtained sequences were deposited in GenBank under the accession no. OM368442, MT899187 and MT900590 respectively. Manasa et al. (2020) identified the fungal OTUs by amplifying the ITS region of the genomic DNA using ITS1 and ITS4 as forward and reverse primers respectively. The molecular identification was reconfirmed by their macro- and micro-morphological characteristics (Fig. 2). The colony of *P. funiculosum* was greyish green with funiculose texture on PDA media and examined biverticillate condiophore with subterminal branches and ellipsoidal conidia. In case of *C. lacerate*, white fluffy colonies was observed with aseptate hyphae. Initially colourless colony was observed in *E. endophytica* and later it become hyaline with shine black color and examined pycnidial conidiomata with ellipsoidal conidia.

Effects of endophytes isolated from cold desert on imparting thermotolerance in rice

High temperature is one of the most important environmental stresses which severely affect the rice growth by reducing the emergence of leaves and tillers resulting in decreased biomass. The fungal endophytes inoculation significantly ($P < 0.01$) improved all growth attributes of rice plants except plant height under both heat stress as well as normal conditions (Table 2 and 3). An endophyte *P. funiculosum* colonized plants found superior in increasing plant height, number of tillers and leaves, root volume, fresh and dry weight of shoot and root in normal growth condition. Whereas under stress condition, significant higher tiller number was recorded when the plants inoculated with *E. endophytica,* which might positively influenced the new tillers under heat stress by reducing the effects of heat stress on tiller bud. This is in accordance with Vila-Aiub et al. (2005) who reported that *Neotyphodium* sp. infected rye grass produced more tillers than uninfected plants. The endophyte *P. funiculosum* inoculated plants showed highest number of leaves compared to other endophytes which resulted in increased fresh weight of shoot. The root system plays a vital role in adaptation of whole plant under heat stress (Huang et al. 2012). Significant improved in root growth was observed in endophytes colonized plants which lead to improved absorption of nutrients and water from soil, resulting in a more vigorous plant and helps to cope of heat stress. *E. endophytica* was again found better in influencing the root growth compared to others. Our results are in agreement with Waqas et al. (2015) who demonstrated that *Paecilomyces formosus* LWL1 improved root biomass of rice under heat stress.

Categories of treatments based on their performance in normal and stress conditions

The treatments were divided into four categories based on Fernandez (1992) model using stress tolerance index of biomass. The treatment *E. endophytica* inoculated plants belongs to group A that indicates the production of higher biomass under the both conditions (normal and stress). The *P. funiculosum* and *C. lacerate* fall under group B having maximum biomass only under normal growth condition. The uninoculated plants formed group D produced least biomass under both the conditions (Fig. 3).

Declarations

Acknowledgments

Authors are grateful to Dr. Uma shaanker, School of Ecology and Conservation laboratory, UAS, GKVK, Bangalore for providing fungal cultures. APS thankful to the Department of Science and Technology (DST), GOI, New Delhi, for awarding INSPIRE fellowship (DST/INSPIRE Fellowship/2016/IF160469).

References

1. Baker JT, Allen LH, Boote KJ (1992) Temperature effects on rice at elevated CO2 concentration. J Exp Bot 43:959–964. https://doi.org/10.1093/jxb/43.7.959
2. Baldani VD, Baldani JI and Döbereiner J (2000) Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillumseropedicae* and *Burkholderia* spp. Biol Fertil Soils 30:485–491. https://doi.org/10.1007/s003740050027
3. Bhunjun CS, Phillips AJL, Jayawardena RS, Promputtha I, Hyde KD (2020) Importance of Molecular Data to Identify Fungal Plant Pathogens and Guidelines for Pathogenicity Testing Based on Koch's Postulates. Pathogens. 10: 10-96. https://doi.org/10.3390/pathogens10091096

4. Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. In: Proceedings of the international symposium on adaptation of vegetable and other food crops in temperature and water stress. Taiwan. pp. 257-270. http://dx.doi.org/10.22001/wvc.72511
5. Huang B, Rachmilevitch S, Xu J (2012) Root carbon and protein metabolism associated with heat tolerance. J Exp Bot 63(9):3455–3465. https://doi.org/10.1093/jxb/ers003
6. Krishnan PB, Ramakrishnan K, Raja Reddy, Reddy VR (2011) High-Temperature Effects on Rice Growth, Yield, and Grain Quality. Advances in Agronomy. 87-209. https://doi.org/10.1016/B978-0-12-387689-8.00004-7
7. Manalo PA, Ingram KT, Pamplona RR, Egeh AO (1994) Atmospheric carbon dioxide and temperature effects on rice. Agric. Ecosyst. Environ. 51: 339-347. https://doi.org/10.1016/0167-8809(94)90145-7
8. Manasa KM, Vasanthakumari MM, Nataraja KN, Uma Shaanker R (2020) Endophytic fungi of salt adapted *Ipomeapes-caprae* L.: their possible role in inducing salinity tolerance in paddy (*Oryza sativa* L.). Curr Sci 118(9):1448-1453. http://dx.doi.org/10.18520/cs/v118/i9/1448-1453
9. Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, et al (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci USA 101: 9971-9975. https://doi.org/10.1073/pnas.0403720101
10. Redman, RS, Sheehan, KB, Stout, RG, Rodriguez, RJ & Henson, JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science. 298 (5598):1581-1581. https://doi.org/10.1126/science.1072191
11. Tewari VP, Kapoor KS (2013) Western Himalayan Cold Deserts: Biodiversity, Eco-Restoration, Ecological Concerns and Securities. *Annals of Arid Zone* 52(3&4): 223-230.
12. Vainio EJ, Korhonen K, Hantula J (1998) Genetic variation in *Phlebiopsisgigsntea* as detected with random amplified microsatellite (RAMS) markers. Mycol. Res. 102:187–192. https://doi.org/10.1017/S0953756297004577
13. Vila-Aiub MM, Gundel PE, Ghersa CM (2005) Fungal endophyte infection changes growth attributes in *Lolium multiflorum* Lam. Austral Ecology 30(1):49-57. https://doi.org/10.1111/j.1442-9993.2005.01423.x
14. Wang F, Zhang R, YuanZ, Chen P (2021) Biological prevention and control of pitaya fruit canker disease using endophytic fungi isolated from papaya. Arch Microbiol. 1-8 https://doi.org/10.1007/s00203-021-02378-4
15. Waqas M, Khan AL, Shahzad Ul, Khan AR, Lee IJ (2015) Mutualistic fungal endophytes produce phytohormones and organic acids that promote japonica rice plant growth under prolonged heat stress. J Zhejiang Univ-Sci B (Biomed & Biotechnol) 16(12):1011-1018. https://doi.org/10.1631/jzus.b1500081
16. Yoshida S (1981). Fundamentals of Rice Crop Science. International Rice Research Institute, Los Banos, Philippines. https://pdf.usaid.gov/pdf_docs/PNAAJ868.pdf
17. Zhang Q, White JF (2021) Bioprospecting Desert Plants for Endophytic and Biostimulant Microbes: A Strategy for Enhancing Agricultural Production in a Hotter, Drier Future. Biology (Basel).10(10): 961. https://doi.org/10.3390/biology10100961

Tables

Table 1 Effects of different temperatures on growth of the fungal colony (diameter in cm)

Endophytic fungal isolates	Temperature (° C)						
	28	**30**	**32**	**34**	**36**	**38**	**40**
A1	6.13±0.23	5.80±0.1	5.76±0.09	2.73±0.12	1.83±0.03	0.6±0.05	-
A2	4.36±0.09	5.67±0.09	4.5±0.17	3.46±0.12	2.83±0.09	1.80±0.06	1.43±0.03
A3	6.03±0.15	5.20±0.12	4.13±0.09	2.80±0.06	3.00±0.12	0.73±0.03	-
A4	4.50±0.06	3.70±0.25	1.73±0.03	-	-	-	-
A5	5.37±0.09	4.00±0.12	2.90±0.06	2.00±0.06	1.32±0.15	-	-
A6	3.76±0.09	2.90±0.06	1.86±0.09	1.20±0.03	-	-	-
A7	5.33±0.33	4.03±0.09	4.36±0.09	3.80±0.12	2.83±0.09	2.03±0.20	1.40±0.06
A8	4.8±0.06	3.03±0.03	1.76±0.09	-	-	-	-
A9	2.30±0.06	1.90±0.06	0.63±0.09	-	-	-	-
A10	3.56±0.21	2.73±0.15	1.86±0.12	1.56±0.21	1.43±0.12	0.86±0.06	-
A11	4.03±0.12	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-
A12	1.90±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-
X1	3.50±0.17	2.93±0.09	2.26±0.09	1.36±0.09	-	-	-
X2	3.66±0.03	2.40±0.06	1.80±0.06	1.33±0.09	-	-	-
X3	5.96±0.09	5.50±0.06	5.00±0.06	4.43±0.03	1.43±0.07	-	-
X4	1.76±0.09	1.26±0.07	-	-	-	-	-
X5	8.76±0.03	8.16±0.03	8.66±0.09	8.06±0.18	3.46±0.09	1.93±0.09	1.13±0.06
X6	3.96±0.09	2.9±0.06	1.86±0.09	1.26±0.07	-	-	-
X7	4.00±0.12	2.93±0.09	2.26±0.09	1.26±0.03	-	-	-
X8	3.93±0.09	2.90±0.06	1.63±0.09	-	-	-	-
X9	1.9±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-
X10	3.63±0.09	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-

Data shown above are the means of three replication with ± standard error. *A- Artemisia plant X- Xerophytic plant*

Table 2 Influence of fungal endophytes on shoot attributes of rice under stress [S] and without stress [WS]

Treatments	Plant height (cm)		No. of Tillers (/3plant)		No. of Leaves (/3plant)		Fresh wt. shoot (g/3plant)		Dry wt. shoot (g	
	WS	S	WS	S	WS	S	WS	S	WS	S
Control	33.53±0.72^{b}	32.76±0.27^{a}	8.50±0.20^{c}	6.50±0.20^{b}	36.50±0.61^{d}	28.00±0.41^{c}	5.95±0.07^{d}	3.13±0.02^{c}	1.54±0.02^{c}	0
C. lacerata	35.51±1.17^{ab}	32.62±0.59^{a}	10.50±0.20^{b}	6.00±0.00^{c}	40.00±0.00^{c}	27.50±0.20^{c}	7.92±0.08^{c}	3.77±0.07^{b}	2.33±0.06^{b}	0
E. endophytica	36.96±0.53^{a}	32.01±0.45^{a}	11.00±0.00^{b}	7.00±0.00^{a}	45.00±0.12^{b}	37.00±0.41^{b}	8.89±0.05^{b}	3.83±0.00^{b}	2.26±0.01^{b}	1
P. funiculosum	36.62±0.19^{a}	32.79±0.76^{a}	14.50±0.20^{a}	6.50±0.20^{b}	55.50±0.20^{a}	39.00±0.41^{a}	10.36±0.08^{a}	4.21±0.05^{a}	2.64±0.02^{a}	1
($F_{3,12}$)	3.31	0.432	199.33	8.00	142.78	263.85	660.86	107.57	184.85	4
P	0.028	0.734	<0.0001	0.003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at $P < 0.05$ by using Duncan's Multiple Range Test.

Table 3 Influence of fungal endophytes on root traits and total biomass of rice under stress [S] and without stress [WS]

Treatments	Root volume (cm^3/3plant)		Fresh wt. root (g/3plant)		Dry wt. root (g/3plant)		Root:Shoot		Biomass (g/3plant)	
	WS	S	WS	S	WS	S	WS	S	WS	S
Control	12.00±0.00^{b}	3.00±0.00^{c}	7.88±0.16^{c}	2.23±0.05^{d}	1.38±0.05^{bc}	0.29±0.01^{d}	0.89±0.02^{a}	0.34±0.01^{c}	2.92±0.06^{c}	1.16±
C. lacerata	6.50±0.00^{d}	3.95±0.10^{b}	7.76±0.55^{c}	3.45±0.00^{c}	1.27±0.06^{c}	0.35±0.01^{c}	0.55±0.02^{d}	0.39±0.01^{b}	3.60±0.12^{b}	1.22±
E. endophytica	11.50±0.20^{c}	4.25±0.02^{a}	9.88±0.15^{b}	4.03±0.06^{a}	1.46±0.02^{b}	0.47±0.02^{a}	0.64±0.01^{c}	0.44±0.02^{a}	3.72±0.03^{b}	1.56±
P. funiculosum	13.90±0.04^{a}	4.00±0.00^{b}	12.73±0.07^{a}	3.64±0.00^{b}	1.98±0.04^{a}	0.39±0.01^{b}	0.75±0.01^{b}	0.36±0.01^{bc}	4.61±0.07^{a}	1.44±
($F_{3,12}$)	920.23	111.39	59.85	445.68	46.17	56.97	101.74	15.35	83.62	319.9
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.000	<0.0001	<0.00

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at $P < 0.05$ by using Duncan's Multiple Range Test.

Figures

Figure 1

MaximumLikelihood tree of the identified fungal endophytes (a) *Penicillium funiculosum* isolate A2) (b) *Ceriporia lacerate* isolate A7 and (c) *Endomelanconiopsis endophytica* isolate X5 and their closest ITS rDNA matches from the GenBank. The phylogenetic tree was constructed with bootstrap value of 500 replicates. Number at the node indicates the bootstrap value.

Figure 2

Fungal colony grown on PDA medium and their fruiting body under microscope (lactophenol cotton blue stain) (a) *Penicilium funiculosum* isolate A2 colony and their conidiophore (b) *Ceriporia lacerate* isolate A7 colony and their aseptate mycelia colony and their hyphae (c) *Endomelanconiopsis endophytica* isolate X5and their mycelia.

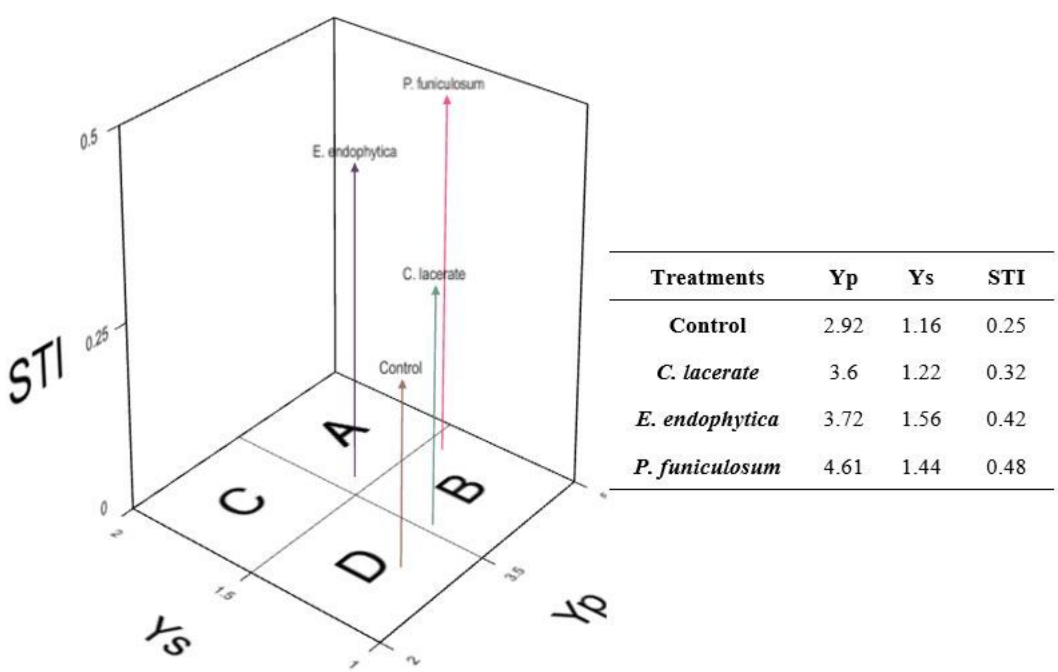

Figure 3

Three dimentional plot based on Fernandez (1992) model using stress tolerance index (STI) of biomass. Yp: Biomass under normal growth condition Ys: Biomass under heat stress.